Aurora Biolabs
High Purity Protein for Research

CDK2/CyclinE1 Kinase Assay Kit

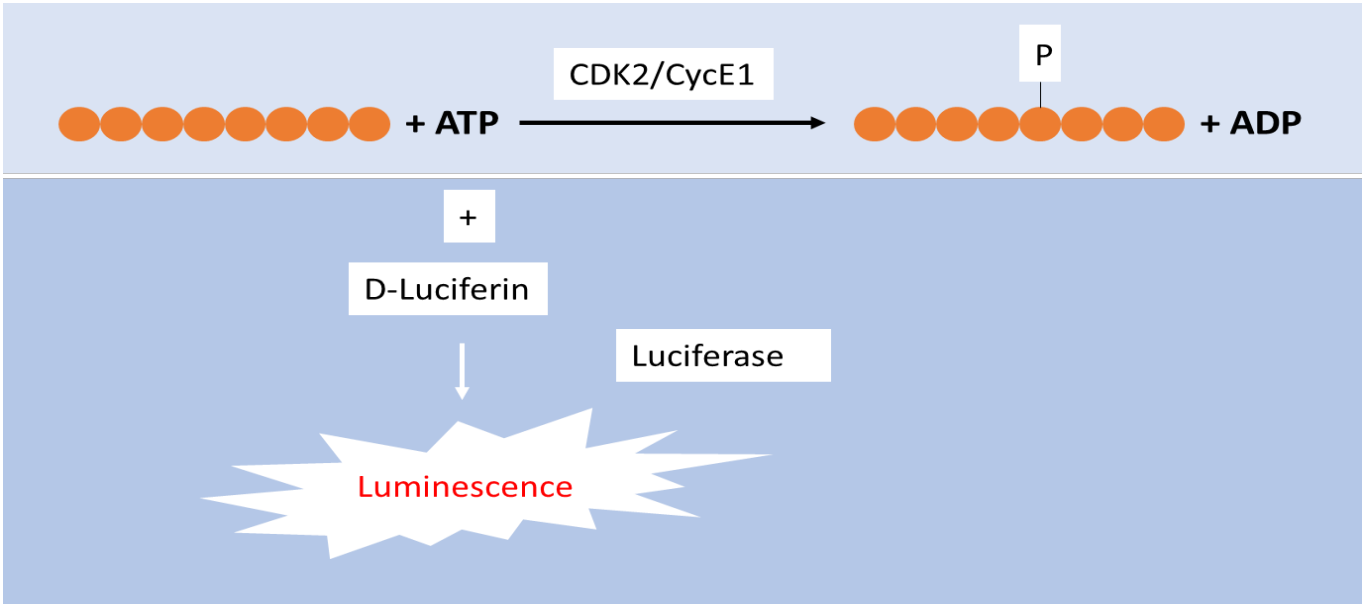
Catalog Number: C352E1-KA

Background

Cyclin-dependent kinase 2 (CDK2) is a member of cyclin-dependent Ser/Thr protein kinase family. It associates with its regulatory subunit cyclin E or cyclin A and plays an important role in controlling the G1/S and S/G2 transitions in the cell cycle. Deregulation of cell cycle is involved in the pathology of many human diseases, including cancers. Given the roles of CDK2 in tumorigenesis, selective CDK2 inhibition may provide therapeutic benefits against certain cancers.

Assay Principle

The CDK2/CyclinE1 assay kit is a luminescence based assay by measuring remaining ATP amount after the kinase reaction using firefly luciferase. The assay kit is designed in 384-well format and is convenient for high-throughput compound screening. CDK2 uses ATP to phosphorylate its substrate peptide to produce the phosphorylated peptide and ADP. Its kinase activity then can be estimated by measuring the amount of the remaining ATP.



Application

High throughput screening of compounds that inhibit CDK2 activity for drug discovery.

Materials needed but not supplied

1. Microplate reader capable of measuring luminescent intensity.
2. Adjustable micro-pipettor
3. Sterile Tips

CDK2/CyclinE1 Kinase Assay Kit

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Components

Catalog number	Item	Amount	Storage
C352KAB	2x Assay Buffer	20 mL	-20°C
C352E1	Recombinant human CDK2/CyclinE1	80 µL	-80°C
C352003	5 mM CDK2 substrate peptide	32 µL	-80°C
BBAB020	1 mM ATP	80 µL	-80°C
SLMB102	D-Luciferin	20 µL	-80°C
PME1701	Firefly luciferase	20 µL	-80°C
	384-well microplate	1	Room temperature

Assay Protocol

1. Prepare 1X buffer

   For example, mix 1000 µl distilled water with 1000 µl of 2X assay Buffer (catalogue number: C352KAB). Store the remaining 2X assay buffer at -20°C.

2. Prepare the inhibitor compound solution

   If the inhibitor compound is dissolved in water, make a solution of the compound 10-fold higher than the final concentration in 1X assay buffer (since you will add 2 µl to the 20 µl reaction).

   If the inhibitor compound is dissolved in DMSO, make a 100-fold higher concentration of the compound in DMSO than the highest concentration you want to test. Then make a 10-fold dilution in 1X assay buffer (at this step, the compound concentration is 10-fold higher than the final concentration and the DMSO concentration is 10%). To determine an IC50 or to test lower concentrations of the compound, prepare as series of further dilutions in 1X assay buffer containing 10% DMSO (the final concentration of the DMSO will be 1% in all samples).

3. Prepare CDK2/CycE1 solution

   Thaw CDK2/CycE1 protein on ice. Upon first thaw, briefly spin tube to recover the full contents at the bottom of the tube. Make aliquots of the enzyme for single use. Store remaining undiluted enzyme at -80°C.

   Note: CDK2/CycE1 protein is sensitive to freeze/thaw cycles. Limit number freeze-thaw cycles for best results. Do not re-use the diluted protein.

Dilute the CDK2/CycE1 protein 40-fold using 1X assay buffer. For example: 1 µl of CDK2/CycE1 + 39 µl of 1X assay buffer.

Add 8 µl of diluted protein solution to each of positive control well and inhibitor test well.

Add 8 µl of 1X assay buffer to each of negative control well.

4. Add inhibitor

   Add 2 µl of diluted compound solution to each inhibitor test well.

   Add 2 µl of inhibitor solvent solution to each of positive and negative control wells.

   Incubate at room temperature for 30 minutes (optional).

5. Prepare substrate solution

   Dilute ATP solution 1:50 and dilute CDK2 substrate 1:125 in assay buffer. For example: 5 µl of 1 mM ATP + 2 µl of 5 mM CDK2 substrate + 243 µl of 1X assay buffer.

   Add 10 µl of the substrate solution to each well. The final concentration for both ATP and CDK2 substrate is 10 µM.

6. Incubate at 30°C for 1 hour
7. Prepare luciferase solution

   Dilute luciferase and luciferin 1:200 in 1X assay buffer. For example: 1 µl of luciferase + 1 µl of luciferin + 198 µl of 1X assay buffer.

   Add 10 µl of luciferase solution to each well.

8. Incubate the reaction at room temperature for 15 minutes.
9. Measure luminescent intensity

Protocol Summary

Component	Background	Positive Control	Inhibitor Test
1X buffer	8 µl		
CDK2/CycE1		8 µl	8 µl
Inhibitor solvent	2 µl	2 µl	
Inhibitor solution			2 µl
	10 µl	10 µl	10 µl
Incubate at room temperature for 30 minutes (optional).			
Substrate solution	10 µl	10 µl	10 µl
Total Volume	20 µl	20 µl	20 µl
Incubate at room temperature for 1 hour.			
Luciferase solution	10 µl	10 µl	10 µl

Total Volume	30 µl	30 µl	30 µl
Incubate at room temperature for 15 minutes.			
Measure luminescent intensity.			

Data Analysis

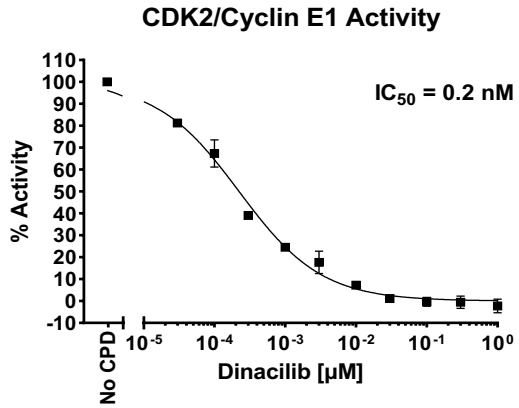
A. Calculate percentage activity

1) Subtract the luminescent Intensity of background well(s) from that of positive and inhibitor test wells.
2) Convert the negative number to positive numbers by times all of numbers with -1.
3) the absence of the compound (positive control), the sample signal (P) is defined as 100% activity. In the absence of enzyme and compound (negative control), the sample signal (N) is defined as 0% activity. The percent activity in the presence of each compound is calculated according to the following equation: % activity = (S-N)/(P-N) X100, where S= the sample signal in the presence of the compound.

$$\%\ \text{activity} = \frac{S - N}{\mathrm{P} - \mathrm{N}} X100$$

Plot the percentage activity against compound concentrations on a graph

Data Presentation



Related products:

Catalog #	Product Name	Size
C352A2-KA	CDK2/Cyclin A2 Kinase Assay Kit	384 Reaction
C352A2	GST-CDK2:His-CyclinA2	10 µg
C352E1	GST-CDK2:GST-CyclinE1	10 µg

This product is for research use only and not for diagnostic or therapeutic use.